

Effect of Sanitizers and Disinfectants in *Staphylococcus Saprophyticus*

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Abstract

Background/Objectives: In the dental environment, handpiece or ultrasonic scaler used in treatment causes aerosols and various pathogenic microorganisms. Many types of pathogens cause infectious diseases. This study is to identify surface disinfectants suitable for the management of *Staphylococcus saprophyticus* bacterial species, one of the causes of infection in medical institutions to use them as standardized surface management resources for infection control in dental clinic.

Method/Statistical Analysis: Commonly used 7 types of disinfectants were classified to select commercialized disinfectants. Samples were taken by rubbing the surface of the unit chair with a sterile swab. In order to check the bacterial species cultured in the medium, a single colony was purely separated by streaking. To identify the bacterial species, DNA was extracted from the bacteria and PCR was performed. the mean and standard deviation of each group. One-way ANOVA and Scheffe's post-test were carried out to identify the clear zone of paper discs and significant differences between groups.

Findings: The test for bacterial identification was carried out for the surface flora of the unit chair. As a result, *S. saprophyticus* was identified on the surface. According to the result of comparative analysis of the clear zone size of each disinfectant, sodium hypochlorite (NaOCl), a chlorine disinfectant was found to have the highest disinfection effect (8.52mm), followed by Distell, an ammonium compound disinfectant (2.72).

Improvements/Applications: The result of the analysis of the clear zone of the disinfectant showed excellent antibacterial activity in 0.3% NaOCl and 0.5% Distel, indicating that they are disinfectants suitable for *S. saprophyticus* used in the study.

Keywords: *Bioaerosol, Disinfectant, Sanitizer, Staphylococcus saprophyticus, Unit chair.*

Introduction

In the dental environment, handpiece or ultrasonic scaler used in treatment causes aerosols and various pathogenic microorganisms^[1]. Many types of pathogens

caused by handpiece treatment using the spraying force of compressed air are likely to cause infectious diseases by floating the treatment space and falling to the surface of the dental equipment^[2]. Staphylococci are Gram-positive cocci that are present in the mucous membranes of the skin and oral throat, and are known to be the main bacteria of purulent inflammation^[3]. Multidrug-resistant bacterial infections called superbacteria are particularly deadly among infections in medical institutions because treatment is difficult. Among them, the typical methicillin-resistant *Staphylococcus aureus* requires further management because it has an infection rate of 62-66% in hospitals and 46-49% in clinics^[4]. Present on human mucous membranes and skin as normal bacteria,

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Staphylococcus aureus not only causes toxin-type food poisoning caused by enterotoxin, but also causes various purulent infections and fatal sepsis, which account for more than 80% of the purulent diseases in the treatment^[5]. In some cases, infections in soft tissues, salivary glands, extraction sites, and alveolar bones may expand to the facial area. Therefore, the importance of *staphylococcus aureus* infection control is very emphasized. Among staphylococci of the same series, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* (*S. saprophyticus*), which are problematic in clinical trial, are known to be infectious bacteria in hospitals and are at risk in invasive medical devices or in groups with low immunity and sometimes appear in urinary tract infections in young women^[6-7].

In order to manage infection in the dental environment, the Ministry of Health and Welfare implemented a certification system for dental hospitals in 2014 and presented the legislation of medical institution evaluation and infection control standards^[8]. In the United States, the Centers for Disease Control and Prevention (CDC) recommends disinfecting the surface of the unit chairs such as examining tables, switches, and lamp handles contaminated by aerosol formation, etc. after each patient's treatment, or using a protective cover in case of visible contamination. Infection control refers to a management method to prevent or reduce the causes of infection in medical institutions in order to protect patients, staff, visitors and other environments^[9], and various types of disinfectants and sterilizers are used for infection control in dental clinic. Disinfection is an operation that removes the risk of infection by destroying the viability of pathogenic microorganisms using relatively weak sterilizing power and that kills some of the microorganisms, and sterilization is the process of destroying all types of organisms, including gemmula, using physical and chemical method and means complete sterility^[10]. As disinfection and sterilization method commonly used in dental clinics, the autoclaving method is commonly carried out for metal equipment for dental treatment, and chemical disinfection such as glutaraldehyde, physical disinfection such as ultrasound and ultraviolet disinfection are conducted for rubber and plastic materials^[11]. However, accurate cleaning, disinfection and sterilization are important because improper disinfection and sterilization are more likely to lead to cross infections. Jeong et al.^[12] emphasized the importance of sterilization and disinfection method suitable for instruments and equipment by presenting the

research findings that the contamination of bacteria was very high when the surface around the unit chair was not disinfected and pathogens were further spread from the cause of infection when the surface disinfectant was not applied with the proper disinfection method.

Thus, the purpose of this study is to identify surface disinfectants suitable for the management of *S. saprophyticus* bacterial species, one of the causes of infection in medical institutions to use them as standardized surface management resources for infection control in dental clinics.

Method

Samples were taken by rubbing the surface of the unit chair with a sterile swab. 100 µL of each collected sample was dispensed to premade LB medium (Becton; Dickinson and Company, Sparks, MD, USA) and spreading was performed with a sterile glass rod. They were cultured in a culture medium for 24 hours at 37°C and the number of colonies was measured. In order to check the bacterial species cultured in the medium, a single colony was purely separated by streaking. To identify the bacterial species, DNA was extracted from the bacteria and PCR was performed.

S. saprophyticus (KCCM 41662) was purchased from Korean Culture Center of Microorganisms (KCCM). Each microorganism was activated by tryptic soy broth (TSB; BD, Sparks, MD, USA) with 5% sheep blood and was diluted at a 2x10⁶ ratio.

Commonly used 7 types of disinfectants were classified to select commercialized disinfectants. The disinfectants were prepared and used in the ratio shown in [Table 1] immediately before the test as recommended by the manufacturer.

Table 1. List of sanitizer and disinfectant in this study

Variables	Concentration (%)
PBS	100
Alcohol	70
Hydrogen peroxide	3
Sodium Hypochlorite	0.3
Y-Na Solution	2
Distel	0.5
Eco rich	100

100 µL (2x10⁶) of *S. saprophyticus* was applied on a solid LB medium. 30 µL of each experimental group

was dropped onto a paper disc, and it was placed on the LB medium inoculated with the bacteria. After keeping it at 37°C for 24 hours in each environment, the diameter of the clear zone was measured on a paper disc. The average value and standard deviation were obtained after three repeated experiments, to measure the diameter of the clear zone, where the growth was inhibited. The average value of clear zone was calculated, and used for comparative analysis in the results.

SPSS 24.0 program was used for the statistical analysis. Descriptive statistics were used to obtain the mean and standard deviation of each group. One-way ANOVA and Scheffe’s post-test were carried out to identify the clear zone of paper discs and significant differences between groups.

Result and Discussion

1. General characteristics of the bacterial species:

In this study, microorganisms were collected from the surface of the unit chair to measure the contamination caused by these floating bacteria. The test for bacterial identification was carried out for the surface flora of the unit chair. As a result, *S. saprophyticus* was identified on the surface [Table 2].

Table 2. Characterization of *S. saprophyticus*

Family	Staphylococcaceae
Genus	Staphylococcus
Species	Staphylococcus saprophyticus

2. Antimicrobial Activity: Seven disinfectants were applied to *S. saprophyticus* and as a result, the largest clear zone was found in sodium hypochlorite [Figure 1].

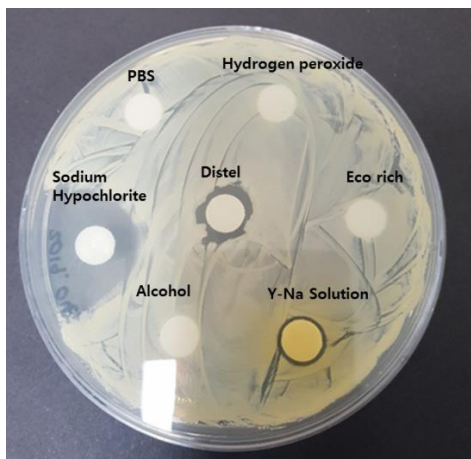


Figure 1. Comparison of death zones by disinfectant

According to the result of comparative analysis of the clear zone size of each disinfectant, sodium hypochlorite (NaOCl), a chlorine disinfectant was found to have the highest disinfection effect (8.52mm), followed by Distell, an ammonium compound disinfectant (2.72) [Table 3].

Table 3. Values of death zone by sanitizer and disinfectant

Variables	Mean	SD	p-value
Control(PBS)	0.00	0.00	0.000***
Alcohol	0.00	0.00	
Hydrogen peroxide	0.00	0.00	
Sodium Hypochlorite	8.51	0.07	
Y-Na Solution	1.14	0.12	
Distel	2.72	0.70	
Eco rich	0.00	0.00	

***: p<0.01

Discussion

As the importance of hospital infections is highlighted in recent years, there is a growing interest in problems with infection control systems in dental clinics. In dental clinics where bioaerosol is generated such as handpieces and ultrasonic scalers, bio aerosols float in the air drop and cause contamination, causing infectious diseases by dental medical instruments^[13-16]. Various instrument surfaces in the dental clinics also warn that infection can be a source of contamination and a source of contamination^[17].

Staphylococcal infections are known as the main infectious organisms, not limited to their area of occurrence. In particular, affecting groups with weak immunity, it is most problematic in neonatal rooms, intensive care units, operating rooms, etc. and sometimes causes community infections by discharge patients^[18-19]. Among them, *S. saprophyticus* is 5 ~ 8 MM in diameter, very glossy, opaque, smooth and convex.

It has been suggested that the water quality of the unit chair is an important issue for both patients and dental staff because it is exposed to water and aerosols derived from the unit chair^[20]. In addition, various previous studies have identified that *S. saprophyticus* is often included among the microorganisms isolated from the unit chair water line^[21]. Based on this finding, water and aerosols in the form of *S. saprophyticus* are believed to be produced in dental clinics. Previous studies have

recommended proper disinfection and sterilization to avoid infection, and experts emphasized the need to follow standard procedures such as risk assessment, patient protection, sterilization and disinfection^[22-23]. Therefore, various disinfectants were applied to find an appropriate method for disinfecting microbial strains.

Disinfection is the process of killing all pathogenic microorganisms except for the spores of bacteria on the surface of the object. It is usually done using liquid chemical disinfectants and the level and duration of the disinfectant is determined depending on the degree of disinfection of the treatment equipment^[24].

NaOCl releases hypochlorous acid to inhibit enzymatic activity in cells and denatures cell proteins, resulting in bactericidal effects. The guidelines of the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) recommend 0.1% NaOCl (1,000 ppm effective chlorine) for environmental disinfection to prevent the propagation of pathogenic microorganisms^[25]. In this study, 0.3% NaOCl was used according to the manufacturer's instructions. Used at a higher concentration than IDSA, it was considered to show significantly higher killing effect. Among the various disinfectants, the use of chlorine disinfectants was found to be high due to the economic aspects and convenience of use^[26].

The second most effective disinfectant was found to be 0.5% Distel. This disinfectant belongs to the quaternary ammonium disinfectant. Characterized by weak alkali, the quaternary ammonium disinfectant is known to be widely used as a skin disinfectant, preservative, and preserved agent because of its ability to inhibit bacterial culture and sterilization^[27]. In addition, the sterilization effect is excellent in all the pH range, it is a stable disinfectant due to the relatively small influence by temperature.

The third effective disinfectant was 1.14 in 2% Y-Na solution. The most commonly used disinfectant in the world is most effective when used as a 2% buffer solution at pH 7.5-8.5. It can be used as a high level disinfectant and sterilizer for all Gram-positive and Gram-negative bacteria, fungi, viruses (including HBV and HIV) and bacterial spores, including tuberculosis^[28]. However, there is a possibility that toxic substances can be generated when absorbed into plastic products of unit chair, so it is known that caution is required. It is

important to avoid spraying and use personal protective equipment.

In order to carry out higher quality care in addition to the development of dentistry, research on infection control and cross-infection prevention should be carried out continuously. In order to develop a systematic infection control program in Korea, it is necessary to introduce more strict foreign standards for appropriate management for each situation

Conclusion

In this study, *S. saprophyticus*, which affects the surface contamination and causes opportunistic infections, was used to analyze the antimicrobial activity of several disinfectants. The result of the analysis of the clear zone of the disinfectant showed excellent antibacterial activity in 0.3% NaOCl and 0.5% Distel, indicating that they are disinfectants suitable for *S. saprophyticus* used in the study. Based on the above results, a more effective and systematic surface management system is required by analyzing selective killing disinfectants of strains causing specific surface contamination.

Ethical Clearance: Not required

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Conflict of Interest: Nil

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